

# Protection effects on fish assemblages, and comparison of two visual-census techniques in shallow artificial rocky habitats in the northern Adriatic Sea

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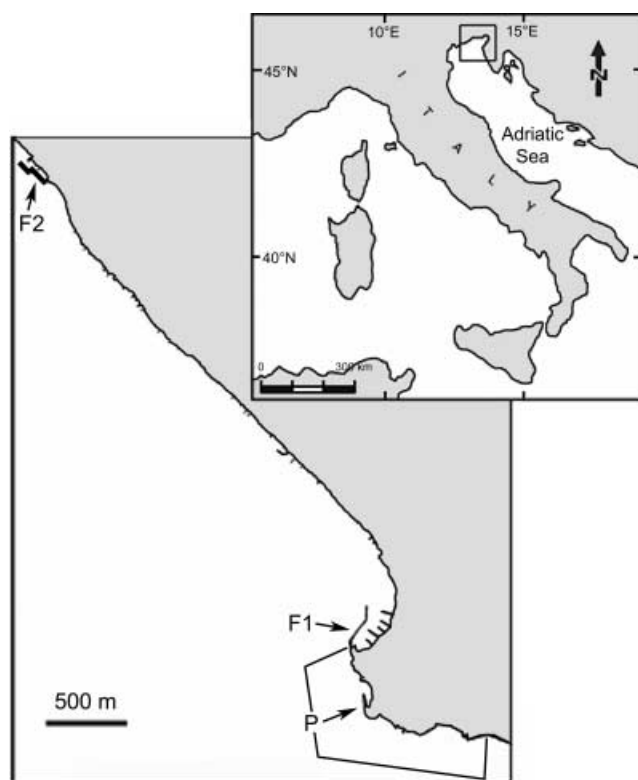
Fish assemblages associated with shallow (4–7 m deep) artificial rocky habitats (i.e. breakwaters) have been assessed between July 2002 and September 2003, at the marine protected area of Miramare and adjacent areas outside the reserve (northern Adriatic sea). Our purpose was to: (1) detect possible differences between ‘protected vs fished’ breakwaters; and (2) compare two visual-census techniques for fish assessment (i.e. strip transects vs stationary points). The fish assemblages observed between protected and fished breakwaters during all four sampling periods were statistically different. More fish taxa were found at the protected than fished breakwaters, while there was no difference in total fish density. Most fish species targeted by fisheries had a greater density (e.g. *Sciaena umbra*, *Dicentrarchus labrax*, *Sparus aurata*, *Diplodus vulgaris*, *Diplodus sargus* and *Diplodus puntazzo*) and/or size (e.g. *S. aurata* and *D. annularis*) at the protected than fished breakwaters. There was a significant difference in fish assemblages due to assessment method. In general, the number of taxa was greater when assessed by strip transects than stationary points. Total fish density was almost unaffected by the method used, while total density of demersal fish (i.e. excluding schooling species) tended to be greater when evaluated by strip transects, although the difference was statistically significant only in one sampling period out of four. These results indicate that protection from fishing may have the potential to influence fish assemblages associated with breakwaters. Additionally, caution should be used when comparing fish assemblage data collected by different visual assessment techniques.

## INTRODUCTION

Marine Protected Areas (MPAs) have become a popular tool for the conservation of marine ecosystems and fishery management (Dayton et al., 2000; National Research Council, 2001). Willis et al. (2003) argued that many theoretical papers, meta-analyses and reviews have been published about the ‘potential’ of MPAs for restoring fish populations, whereas empirical studies about their actual effectiveness are still scanty. This is particularly true in the Mediterranean Sea, where studies evaluating fish responses to protection are restricted to MPAs in the western sector of the basin (e.g. Harmelin et al., 1995; Vacchi et al., 1998; Garcia-Charton et al., 2004).

The Miramare MPA is located in the northern Adriatic Sea, and is the only Mediterranean reserve where rocky habitats are mostly formed by breakwaters. Previous studies in the Miramare MPA reported fish species lists (Castellarin et al., 2001), preliminarily showed the responses of some fish species to protection (De Girolamo et al., 1998), or investigated the relationship between fish predators and sea urchins (Guidetti et al., 2005). No robust evaluation of the effect of protection on fish assemblages, however, has been done so far in the eastern Mediterranean basin and the Adriatic Sea, neither have studies been conducted to assess protection benefits on fish associated with coastal defences, such as breakwaters.

Visual-census methods to assess fish have been widely employed in the Mediterranean Sea to investigate the association between fish and habitats (Harmelin, 1987; Guidetti, 2000; Bussotti et al., 2002), the social organization and reproductive patterns (De Girolamo et al., 1999; Verginella et al., 1999), human impacts (Guidetti et al., 2003), and distribution patterns of juvenile fish (Vigliola et al., 1998). Their use, however, has long been suggested for assessing fish within MPAs, due to their non-destructive nature (Harmelin-Vivien et al., 1985; Harmelin, 1987). Fish assessments in Mediterranean MPAs have been performed chiefly in rocky habitats. Strip transects are the most popular technique (Vacchi et al., 1998; Garcia-Charton et al., 2004, and references therein), where the observer swims along paths of prescribed length and width (and, in some cases, height), and notes species, number and size of fish. In a few cases, stationary points have been employed (Vacchi & Tunesi, 1993; Francour, 1994; Micheli et al., in press), where the observer remains stationary in the centre of a circular area of pre-established radius (and, in some case, height), while recording species, number and size of fish. Details about the two above mentioned techniques of visual-census can be found in Harmelin-Vivien et al. (1985). Although most authors have used strip transects for assessing rocky-reef fish in Mediterranean MPAs, a scant effort has been done to quantitatively compare the two techniques to date, and



**Figure 1.** Study area and location of the protected (P) and fished (F1, F2) breakwaters at the marine protected area of Miramare.

their current use could merely reflect a researcher preference.

This paper, therefore, is directed toward: (1) comparing fish assemblages associated with shallow (4–7 m) protected and fished breakwaters within and outside the MPA of Miramare; and (2) comparing two visual-census techniques for fish assessment, i.e. strip transects and stationary points.

## MATERIALS AND METHODS

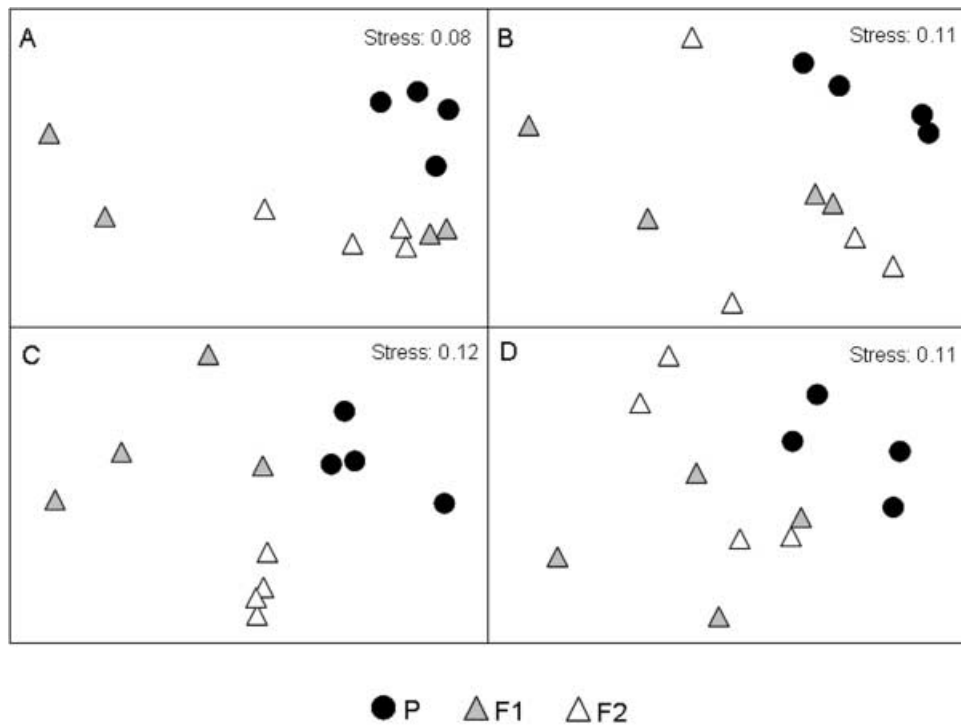
### *Study area, sampling design and data collection*

This study was conducted at the MPA of Miramare and adjacent areas (northern Adriatic Sea, Italy; Figure 1). Miramare is a small reserve (about 121 ha) established in 1986, where rule enforcement has been effective and poaching negligible. Within the MPA, the foreshore is formed by natural and artificial rocky substrates. Artificial structures are formed by external breakwaters (made of transplanted boulders), running parallel to the coast, with internal seawalls.

To assess effects of protection on fish assemblages, fish were visually sampled on breakwaters both inside and outside the MPA, as proper natural rocky substrates outside the MPA were not available. Assessments were conducted at the single protected breakwater within the MPA (hereafter named P), whereas two breakwaters (F1 and F2=F<sub>s</sub>) were assessed for the fish assemblage outside the reserve (Figure 1). Both fished breakwaters are located north of the protected area, as comparable fished

**Table 1.** List of fish taxa (+, present; –, absent) recorded at each of the three breakwaters investigated: P, protected breakwater; F1 and F2, fished breakwaters; \*, target fish taxa.

Family Species	P	F1	F2
Atherinidae (unidentified)	+	+	+
Belonidae			
<i>Belone belone</i>	+	–	–
Blenniidae			
<i>Aidablennius sphynx</i>	+	+	–
<i>Lipophrys dalmatinus</i>	–	+	–
<i>Parablennius gattorugine</i>	+	+	+
<i>Parablennius rouxi</i>	+	+	+
<i>Parablennius sanguinolentus</i>	–	–	+
<i>Parablennius tentacularis</i>	+	+	–
Centracanthidae			
<i>Spicara smaris</i>	–	–	+
Congridae			
<i>Conger conger</i> *	–	–	+
Clupeidae			
<i>Sardina pilchardus</i>	–	–	+
Gobiidae			
<i>Gobius auratus</i>	+	+	–
<i>Gobius bucchichii/fallax</i>	+	+	+
<i>Gobius cobitis</i>	+	+	+
<i>Gobius cruentatus</i>	+	+	+
<i>Gobius niger</i>	+	–	+
<i>Gobius xanthocephalus</i>	+	+	+
<i>Pomatoschistus</i> sp.	+	+	+
Labridae			
<i>Labrus merula</i>	+	+	+
<i>Symphodus cinereus</i>	+	+	+
<i>Symphodus mediterraneus</i>	+	+	+
<i>Symphodus melops</i>	–	–	+
<i>Symphodus ocellatus</i>	+	+	+
<i>Symphodus roissali</i>	+	+	+
<i>Symphodus rostratus</i>	+	+	+
<i>Symphodus tinca</i>	+	+	+
Moronidae			
<i>Dicentrarchus labrax</i> *	+	–	+
Mugilidae (unidentified)*	+	+	+
Mullidae			
<i>Mullus surmuletus</i> *	+	–	+
Pomacentridae			
<i>Chromis chromis</i>	+	+	+
Sciaenidae			
<i>Sciaena umbra</i> *	+	+	+
Scorpaenidae			
<i>Scorpaena porcus</i> *	+	–	–
Serranidae			
<i>Serranus hepatus</i>	+	+	+
<i>Serranus scriba</i> *	+	+	+
Sparidae			
<i>Boops boops</i> *	+	+	+
<i>Dentex dentex</i> *	+	–	–
<i>Diplodus annularis</i> *	+	+	+
<i>Diplodus puntazzo</i> *	+	+	+
<i>Diplodus sargus</i> *	+	+	+
<i>Diplodus vulgaris</i> *	+	+	+
<i>Lithognathus mormyrus</i> *	+	+	–
<i>Oblada melanura</i> *	+	+	+
<i>Sarpa salpa</i> *	+	+	+
<i>Sparus aurata</i> *	+	+	–
<i>Spondylisoma cantharus</i> *	+	+	+
Syngnathidae			
<i>Hippocampus guttulatus</i>	+	–	+
<i>Syngnatus acus</i>	–	–	+
Tripterygiidae			
<i>Tripterygion delaisi</i>	+	+	+
<i>Tripterygion tripteronotus</i>	+	+	+



**Figure 2.** Two dimensional nMDS ordinations of individual replicates comparing fish assemblages at the protected (P) and fished (F1, F2) breakwaters, in each of the four sampling periods (A, T1; B, T2; C, T3; D, T4).

breakwaters were not available southwards. All three breakwaters have similar general features (e.g. wave action) and decline from the water surface to 5–8 m depth over muddy sand.

Reserve effectiveness was evaluated by assessing densities and size of fish by visual-censuses from four to seven metre depths, along transects 25 m long and 5 m wide according with the 'strip transect' method (Harmelin-Vivien et al., 1985). Fish were surveyed in four periods: two random periods from late spring to late summer, both in 2002 and 2003. We selected these sampling periods because, from mid-autumn to mid-spring, few individuals and fish species inhabit shallow rocky habitats in the area (Ota & Odorico, 1993). Fish abundance was

estimated by counting single specimens to a maximum of ten individuals, whereas classes of abundance (11–30, 31–50, 51–100, 101–200, 201–500, >500 individuals) were used for schools. Fish size was assessed by using three size categories (i.e. small, medium, large) on the basis of the maximum total length attained by each species (Fischer et al., 1987). Juvenile stages (settlers and recruits) were not taken into account. Four transects (replicates) were conducted at each breakwater during each sampling period.

To compare 'strip transects' and 'stationary points', further four visual-censuses were performed by using 'stationary points' during each of the four sampling periods, at the protected breakwater. Fish assemblages were assessed from 4–7 m depth, within a 5 m radius (see Vacchi & Tunesi, 1993), using the same criteria for estimating fish density and size. To thoroughly compare fish assessment methods, data were adjusted for the sampling area (Bortone et al., 1989). Both census methods were approximately 8 min in duration.

**Table 2.** One-way ANOSIM testing for differences in fish assemblage structures among breakwaters (P, protected breakwater; F1 and F2, fished breakwaters) in each of the four sampling times (T1, T2, T3, T4; see Materials and Methods).

	T1		T2		T3		T4	
	R value	P	R value	P	R value	P	R value	P
Among locations	0.419	**	0.266	*	0.738	**	0.361	*
Pairwise tests								
F1 vs F2	0.188	n.s.	0.010	n.s.	0.729	*	0.125	n.s.
F1 vs P	0.396	*	0.396	*	0.604	*	0.438	n.s.
F2 vs P	0.771	*	0.417	*	0.979	*	0.594	*

n.s., not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

#### Data analysis

Multivariate techniques were used to analyse fish assemblage structures (PRIMER software package, Plymouth Marine Laboratory, UK; Clarke & Warwick, 1994). Fish density data were logarithm-transformed ( $\ln(x+1)$ ) to reduce weighting given to abundant species. The Bray–Curtis similarity matrix was used to generate two-dimensional ordination plots with the non-metric multi-dimensional scaling (nMDS) technique. An analysis of similarity (ANOSIM) was used to examine differences among breakwaters (Protected vs Fished), and between the two visual techniques (strip transects vs stationary

**Table 3.** SIMPER: fish species contributing most, in percentage (cutting 3%), to the dissimilarity between P and the Fs, during each of the four sampling times (T1, T2, T3, T4; see Materials and Methods).

T1		T2		T3		T4	
Species	%	Species	%	Species	%	Species	%
<i>Sciaena umbra</i>	8.39	<i>Sciaena umbra</i>	8.10	<i>Sciaena umbra</i>	8.39	Mugilidae	9.65
<i>Boops boops</i>	7.45	<i>Pomatoschistus</i> sp.	6.04	<i>Atherina</i> sp.	6.31	<i>Atherina</i> sp.	7.23
<i>Atherina</i> sp.	6.86	Mugilidae	5.89	<i>Boops boops</i>	6.22	<i>Sciaena umbra</i>	6.86
<i>Pomatoschistus</i> sp.	5.79	<i>Chromis chromis</i>	5.36	Mugilidae	5.69	<i>Sarpa salpa</i>	4.86
<i>Diplodus sargus</i>	4.45	<i>Sarpa salpa</i>	5.02	<i>Chromis chromis</i>	5.16	<i>Symphodus cinereus</i>	4.22
<i>Sarpa salpa</i>	4.28	<i>Diplodus vulgaris</i>	4.81	<i>Diplodus puntazzo</i>	4.62	<i>Chromis chromis</i>	4.19
<i>Oblada melanura</i>	3.81	<i>Dicentrarchus labrax</i>	4.13	<i>Symphodus roissali</i>	4.16	<i>Diplodus vulgaris</i>	4.16
Mugilidae	3.47	<i>Diplodus sargus</i>	3.84	<i>Symphodus ocellatus</i>	3.83	<i>Symphodus ocellatus</i>	4.11
<i>Diplodus puntazzo</i>	3.44	<i>Spondyliosoma cantharus</i>	3.81	<i>Sarpa salpa</i>	3.79	<i>Labrus merula</i>	3.67
<i>Serranus scriba</i>	3.42	<i>Diplodus puntazzo</i>	3.80	<i>Labrus merula</i>	3.41	<i>Oblada melanura</i>	3.45
		<i>Oblada melanura</i>	3.35	<i>Diplodus sargus</i>	3.34	<i>Spondyliosoma cantharus</i>	3.33
		<i>Symphodus tinca</i>	3.34	<i>Pomatoschistus</i> sp.	3.20	<i>Sparus aurata</i>	3.15
				<i>Diplodus annularis</i>	3.06		

**Table 4.** Summaries of ANOVAs testing for effects of protection (P vs F), and between the two fished breakwaters (Fs) at each of the four sampling times (T1, T2, T3, T4).

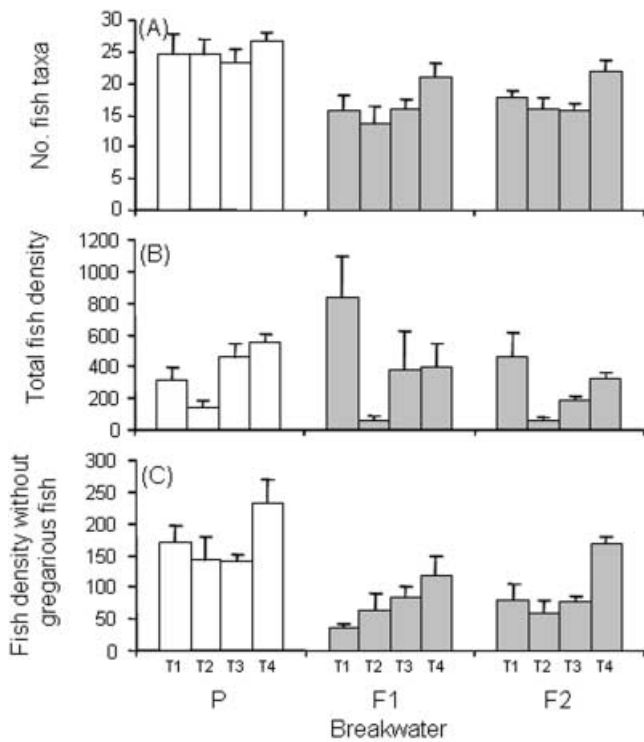
Variable	Source							
	Protection (P vs F)				Between Fs			
	T1	T2	T3	T4	T1	T2	T3	T4
No. of fish taxa	(P>F)*	(P>F)**	(P>F)**	(P>F)*	n.s.	n.s.	n.s.	n.s.
Total fish density	n.s.	(P>F)*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fish density (gregarious species)	(P>F)*	(P>F)*	(P>F)**	(P>F)*	n.s.	n.s.	n.s.	n.s.
<i>Labrus merula</i>	(P>F)**	(P>F)**	(P>F)*	(P>F)**	n.s.	n.s.	n.s.	n.s.
<i>Dicentrarchus labrax</i>	(P>F)*	(P>F)**	—	—	n.s.	n.s.	—	—
Mugilidae	n.s.	n.s.	n.s.	(P>F)**	n.s.	n.s.	n.s.	n.s.
<i>Sciaena umbra</i>	(P>F)**	(P>F)**	(P>F)**	(P>F)*	n.s.	n.s.	n.s.	n.s.
<i>Serranus scriba</i>	(P>F)**	(P>F)**	(P>F)**	n.s.	n.s.	n.s.	n.s.	**
<i>Diplodus annularis</i>	n.s.	n.s.	(P>F)*	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Diplodus puntazzo</i>	—	(P>F)*	(P>F)*	n.s.	—	n.s.	n.s.	n.s.
<i>Diplodus sargus</i>	(P>F)**	(P>F)*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Diplodus vulgaris</i>	—	(P>F)**	n.s.	(P>F)**	—	n.s.	n.s.	n.s.
<i>Sarpa salpa</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Sparus aurata</i>	—	—	(P>F)*	—	—	—	n.s.	—
<i>Spondyliosoma cantharus</i>	(P>F)*	(P>F)*	—	n.s.	n.s.	n.s.	—	n.s.

n.s., not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

points). The similarity percentage analysis (SIMPER) procedure was used to identify the percentage contribution that each species (or taxon) made to the measures of dissimilarity between the average of the Fs vs P, at each sampling period.

Asymmetrical analysis of variance (ANOVA; GMAV5 software package, University of Sydney, Australia) was used to analyse the density of 'relevant' target fish taxa, arbitrarily selected as those contributing to the dissimilarity between protected and fished breakwaters for more

than 3% (SIMPER). The factor 'Protection' (two levels: Protected vs Fished=P vs Fs) was considered as fixed. As there was only one protected breakwater, this is an asymmetrical design (Underwood, 1994). No data were collected before the establishment of the reserve, thus the design is an 'ACI' design (see Glasby, 1997). Asymmetrical designs, their mechanics and potential for detecting temporal and/or spatial differences have been discussed by Underwood (1994) and Glasby (1997). As breakwaters at Miramare (both P and Fs) were too



**Figure 3.** Average ( $\pm$ SE) number of (A) fish taxa; (B) total fish density; and (C) fish density without the numerical of gregarious fish at the protected (P) and fished (F1, F2) breakwaters, in each of the four sampling periods (T1, T2, T3 and T4).

short to randomize the transects during the four sampling periods (i.e. there was overlap among sampling areas during the four sampling periods), independent tests were made for each sampling period to prevent temporal dependence.

Strip transects and stationary points were compared using independent *t*-tests on univariate variables (e.g. number of species, total fish density) for each sampling period.

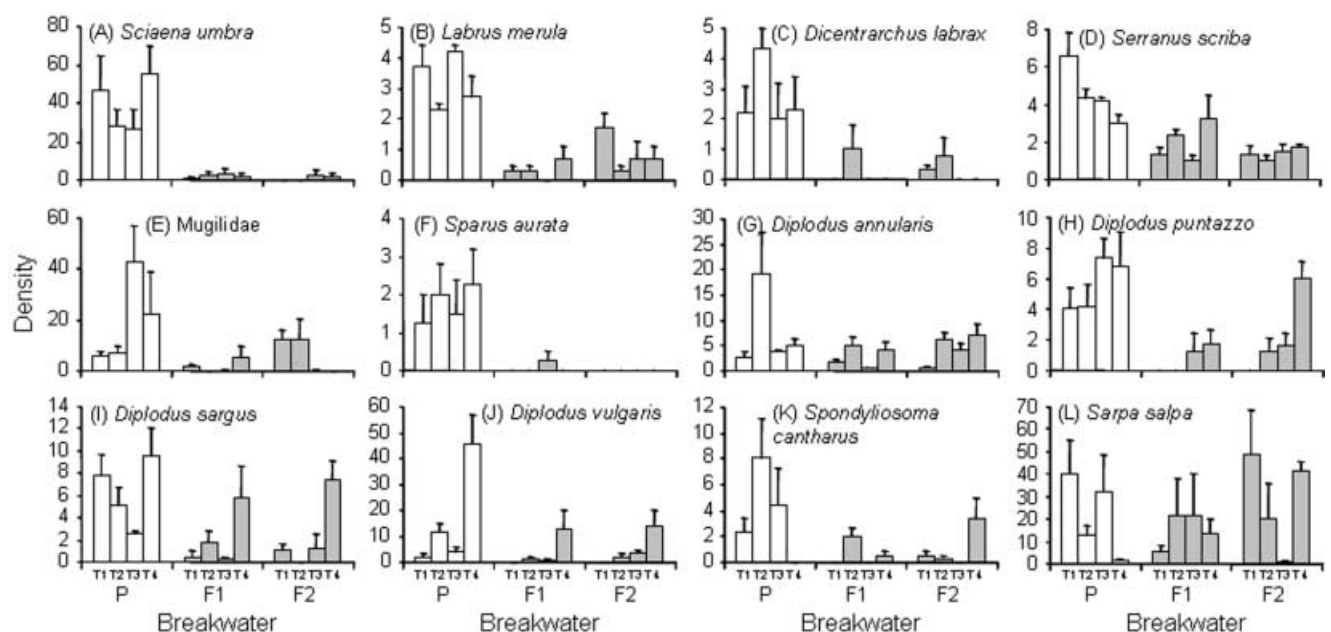
The homogeneity of variances was tested by Cochran's test and, whenever necessary, data were appropriately transformed. Whenever transformations did not produce homogeneous variances, univariate tests were used anyway, after setting  $\alpha=0.01$  to compensate for the increased likelihood of Type I error (Underwood, 1997).

## RESULTS

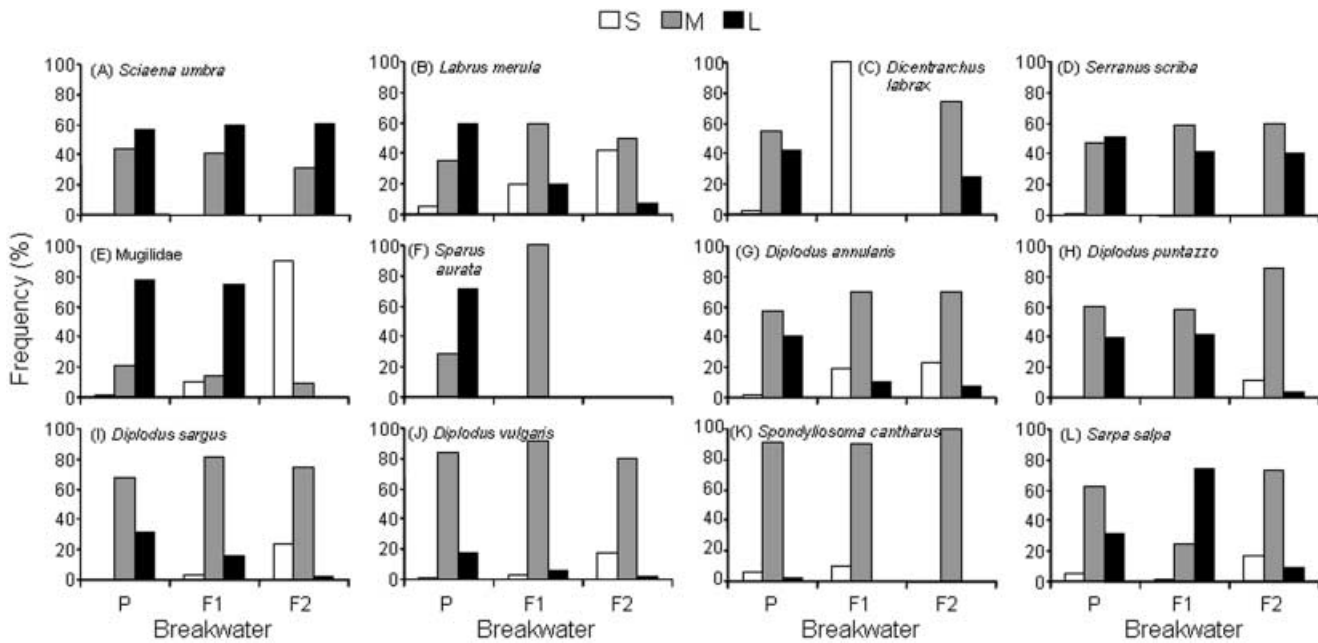
### *Effects of protection on fish assemblages*

Forty-nine fish taxa in 18 families were identified in this study. The taxa recorded at the protected (P: 42 taxa) and fished breakwaters (F1 and F2: 36 and 40, respectively) are reported in Table 1. Mugilids, *Pomatoschistus* spp. and *Gobius bucchichi/fallax* were not identified to the species level due to the difficulties in *in situ* determination.

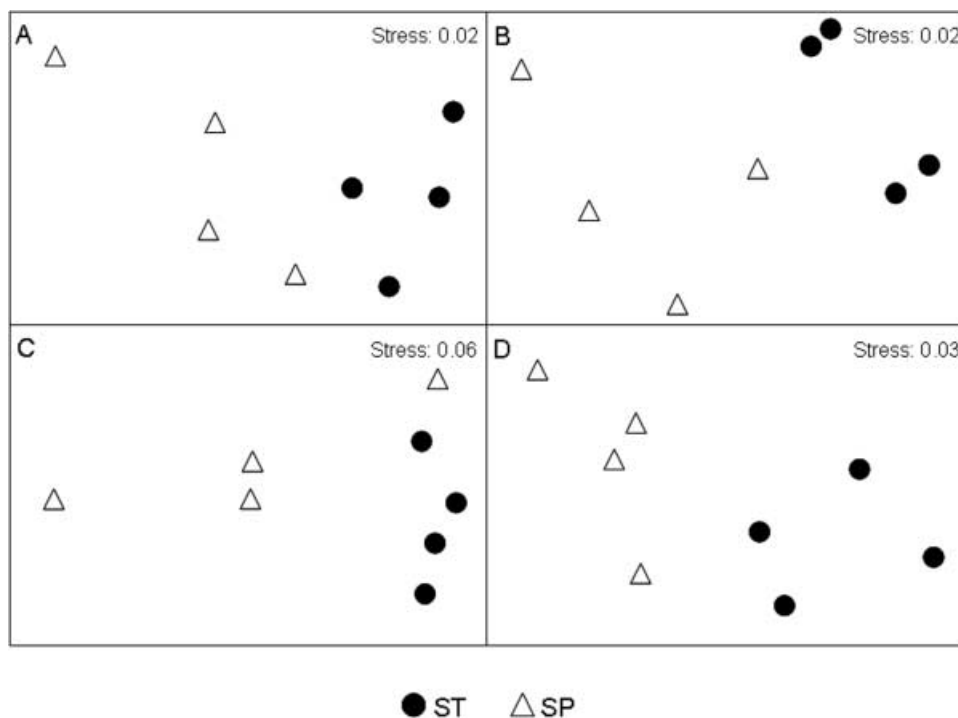
The nMDS plots indicate a clear separation of fish assemblages, during each of the four sampling periods, between Fs and P (Figure 2A–D). One-way ANOSIM indicated that: (1) overall differences in fish assemblages among breakwaters were always significant; (2) differences between the protected and the fished breakwaters were always significant except for 'F1 vs P' in T4; and (3) differences between the two fished breakwaters were always not significant except for T3 (Table 2). The SIMPER identified fish taxa as a major contributor to dissimilarities between P and Fs during each sampling period. *Sciaena umbra* always dominated the censuses at P. Mugilidae, *Diplodus puntazzo*, *D. sargus* (with high density at P), and Atherinidae (mostly associated with Fs) differentiated P from Fs during three sampling periods. *Diplodus vulgaris*, *Spondyliosoma cantharus*, *Labrus merula* (mostly associated



**Figure 4.** Average density ( $\pm$ SE) of 'relevant' target fish taxa (see Materials and Methods) at the protected (P) and fished (F1, F2) breakwaters, in each of the four sampling periods (T1, T2, T3 and T4).



**Figure 5.** Frequency (%) of 'relevant' target fish taxa (see Materials and Methods) at the protected (P) and fished (F1, F2) breakwaters (data of the four sampling times cumulated), in relation to size (S, small; M, medium; L, large).

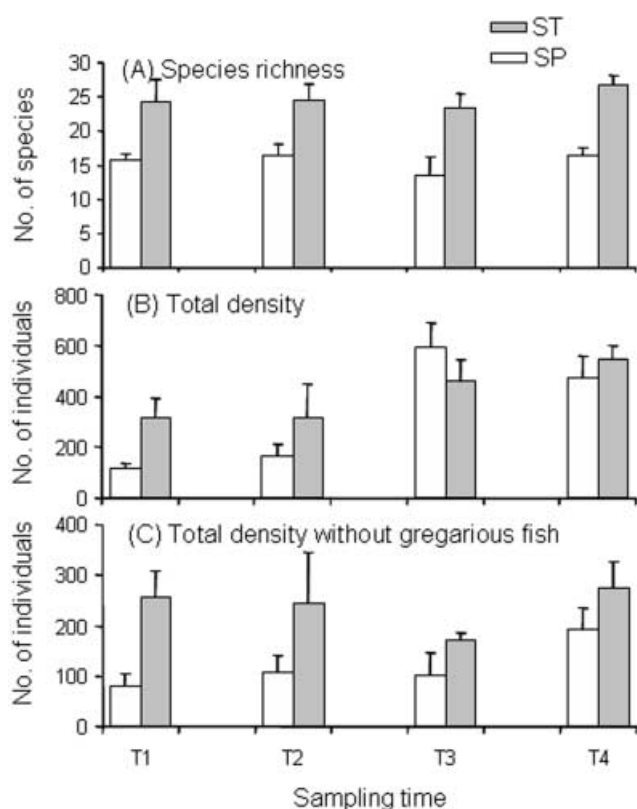


**Figure 6.** Two dimensional nMDS ordinations of individual replicates comparing fish assemblages assessed by strip transects (ST) and stationary points (SP) at the protected breakwater, in each of the four sampling periods (A, T1; B, T2; C, T3; D, T4).

with P), and *Boops boops* (characterizing Fs) differentiated P and Fs during two sampling periods (Table 3).

The results of asymmetrical ANOVAs testing for effects of protection are summarized in Table 4. The number of fish taxa (Figure 3A) was significantly greater at P than

Fs, while no significant variability was detected between Fs throughout the study. Total fish density (Figure 3B) was significantly greater at P than Fs only during T2, while no differences were observed in the remaining sampling periods, or between Fs. Total density of benthic



**Figure 7.** Average ( $\pm$ SE) number of (A) fish taxa; (B) total fish density; and (C) fish density without the numerical contribution of gregarious fish assessed by strip transects (ST) and stationary points (SP) at the protected breakwater, in each of the four sampling periods (T1, T2, T3 and T4).

and necto-benthic fish (i.e. without the numerical contribution of gregarious-planktivorous fish living in the water column; Figure 3C), conversely, was significantly greater at P than Fs, during all four sampling periods, while no differences were observed between Fs. Densities of *Sciaena umbra* (Figure 4A) and *Labrus merula* (Figure 4B) were always significantly greater at P than at Fs, while no variability was detected between Fs. *Dicentrarchus labrax* (Figure 4C) and *Sparus aurata* (Figure 4F) were found only at the protected breakwater during T3 and T4, and during T1, T2 and T4, respectively, while they were more abundant at P than at Fs during the remaining sampling periods.

**Table 5.** *t*-test testing for differences in species richness and total fish density (with and without the numerical contribution of gregarious fish) between techniques (i.e. transect vs point) in each of the four sampling times (T1, T2, T3, T4; see Materials and Methods).

Variable	T1		T2		T3		T4	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
No. of fish taxa	-2.493	0.047*	-2.828	0.030*	-3.565	0.011*	-5.515	0.001**
Total fish density	-2.655	0.037*	-1.094	0.316 n.s.	-1.102	0.312 n.s.	-0.764	0.473 n.s.
Fish density (- gregarious species)	-3.116	0.020*	-1.254	0.256 n.s.	-1.532	0.176 n.s.	-1.257	0.255 n.s.

n.s., not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

*Diplodus vulgaris* (Figure 4J) was found only at P during T1, and was significantly more abundant at P than at Fs during T2 and T4. A significantly greater density of *Serranus scriba* (Figure 4D) was observed during T1, T2 and T3, but not during T4, when a significant variability between Fs was detected. Significantly greater density of *Diplodus sargus* (during T1 and T2; Figure 4I), *D. puntazzo* (during T2 and T3; Figure 4H), *Spondyllosoma cantharus* (during T1 and T2; Figure 4K), *Diplodus annularis* (during T3; Figure 4G) and Mugilidae (during T4; Figure 4E) were observed during some sampling periods at the protected than at the fished breakwaters. There was no difference, conversely, in *Sarpa salpa* between P and Fs, or between Fs, in any of the four sampling times (Figure 4L).

The percentage frequency distributions of the three size-classes in relation to the protection level showed different patterns among fish species (Figure 5A–L). The frequency of large-sized *Labrus merula*, *Sparus aurata* and *Diplodus annularis* (Figure 5B,F,G), and to a lesser extent *Dicentrarchus labrax*, *Serranus scriba*, *Diplodus sargus* and *D. vulgaris* (Figure 5C,D,I,J) was higher at P than Fs. No clear evidence of protection effects on the frequency of the three size-classes, conversely, has been observed for *Sciaena umbra*, Mugilidae, *Diplodus puntazzo*, *Spondyllosoma cantharus* and *Sarpa salpa* (Figure 5A,E,H,K,L).

#### Comparison between strip transects and stationary points

Forty-two fish taxa were recorded by means of strip transects, and 34 by stationary points. The nMDS plots indicating the fish assemblages determined by the two visual-census techniques show a clear-cut separation in each of the four sampling times (Figure 6A–D). One-way ANOSIM revealed that overall differences in fish assemblages were always significant (T1:  $R=0.490$ ,  $P < 0.05$ ; T2:  $R=0.635$ ,  $P < 0.05$ ; T4:  $R=0.844$ ,  $P < 0.05$ ), but in T3 ( $R=0.438$ , n.s.).

The number of fish taxa was significantly greater when assessed by strip transects than stationary points, during each of the four sampling periods (Figure 7A; Table 5). Fish density (with and without the numerical contribution of gregarious fish; Figure 7B,C) was significantly greater when determined by strip transects than stationary points only during T1 (Table 5). The inspection of the graphs, however, reveals that density of demersal fish (i.e. excluding schooling species) tended, in most cases, to be greater when evaluated by strip transects.

## DISCUSSION

The results of this study show that protection significantly affected fish assemblages associated with breakwaters in the northern Adriatic Sea, as well as the number of species, total density of demersal fish (i.e. excluding schooling species), and abundance and size of many target fish, that were greater at the protected than at fished breakwaters.

Literature data suggest that species richness is generally greater in MPAs than in fished areas, while the outcomes concerning total fish abundance are often unclear and display a wide variability among MPAs (Mosquera et al., 2000; Côté et al., 2001, and references therein). The pattern for greater fish density inside MPAs, however, are much clearer when the analyses are restricted to target fish (Mosquera et al., 2000; Côté et al., 2001). This could explain why we found negligible effects of protection on total fish density, while pooled density of necto-benthic fish (that include many target species) was greater within the Miramare MPA than outside, in all four sampling periods. It is well known, in addition, that protection may affect size distribution of fish populations. Larger individuals of target fish are usually more abundant within MPAs than in fished areas, and this general pattern has primarily been attributed to the lack of fishing impact (García-Rubies & Zabala, 1990; Harmelin et al., 1995; Mosquera et al., 2000). However, processes regulating biomass partitioning related to size in fish populations are very complex and may vary not only in relation to the local fishing impact, but also to the habitat type, the local productivity, and the indirect impact caused by changes in trophic interactions among species triggered by the removal of larger-bodied predatory fish (Macpherson et al., 2002; Dulvy et al., 2004).

Several studies from the western Mediterranean basin report that abundance, size and/or biomass of many target fish species are greater within MPAs than in fished areas (Harmelin et al., 1995; La Mesa & Vacchi, 1999; Garcia-Charton et al., 2004, and references therein). At Miramare, a positive response to protection for some fish (i.e. *Sciaena umbra*, *Diplodus vulgaris* and *Dicentrarchus labrax*) was previously reported by De Girolamo et al. (1998), but the sampling design we adopted allowed us to provide more robust evidence of protection effects on fish, stressing once again how important it is to use appropriate sampling designs in similar studies (Guidetti, 2002). The bulk of fish species that significantly responded to protection at Miramare (e.g. *Sciaena umbra*, *D. labrax*, *Diplodus sargus*, *D. vulgaris*, *Sparus aurata* and *Serranus scriba*) are targeted by many kinds of fisheries (e.g. spearfishing, trammel nets, angling; Harmelin, 1987; Harmelin et al., 1995). All the above issues, therefore, suggest that fishing impact may strongly impact target fish, but also that MPAs have the potential to restore depleted fish stocks. Such effects seem to occur also within very small MPAs, such as Miramare, where most of the rocky substrate is formed by breakwaters. Our study thus supports the hypothesis that small reserves may be effective in restoring fish assemblages (Halpern, 2003), and that human-made coastal defence structures could be successfully included within MPAs (Guidetti, 2004).

Strip transects and stationary points produced significantly different fish assemblages. Species richness, in addition, was greater when evaluated using strip transects. The estimates of total density of fish were almost unaffected, while total density of demersal fish (i.e. excluding schooling species) tended to be greater when evaluated by strip transects, although the difference was statistically significant only in one sampling period out of four.

Bortone et al. (1989), evaluating the efficiency of the two methods based on reef fish assessments at Puerto Rico, reported that, once data have been adjusted for survey time and/or sampling area, the transect technique records more species and individuals than stationary points. Bortone et al. (1989) also argued that, using transects, the observer is able to visually concentrate on the area immediately ahead so that there are no complications in trying to detect fish at the limit of the visual acuity, as would be the case when using stationary points. Historically, transects involved preplacement of ropes as reference lines, which is time consuming and potentially causes some bias in the following fish assessments (Bortone et al., 1989). At present, however, this problem has been resolved as divers put down a weight attached to a measuring line (in our case 25 m long) at the beginning of each transect, and the line is then unreeled while censusing fish.

All the above issues and the results of this study support the statement by Bortone et al. (1989), that strip transects remains a preferred technique for quantitatively censusing fish assemblages in homogeneous habitats (in terms of habitat type). Stationary points, however, should be used in highly heterogeneous habitats (e.g. mosaics of different habitats) or in artificial reefs (often constituted by 'discrete units'), where transects may not be feasible (Harmelin-Vivien et al., 1985; Bohnsack & Bannerot, 1986). Harmelin-Vivien et al. (1985), moreover, suggest to use stationary points to assess fish at isolated discrete structures, such as artificial reefs and blocks, and/or for studying restricted pools of species. In contrast, transects should be preferred in homogeneous and extended habitats, and for studying whole fish assemblages. Because of the peculiar features of many artificial reefs, often constituted by pyramids of concrete blocks, D'Anna et al. (1999) also suggested the use of mixed techniques, so to adapt visual-census to each specific context. As a general rule, anyway, the fact that different results are obtained by using strip transects or stationary points suggests the need for caution when comparing data collected by means of different visual-census techniques.

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## REFERENCES

- Bohnsack, J.A. & Bannerot, S.P., 1986. A stationary visual census technique for quantitatively assessing community structure of coral reef fishes. *NOAA Technical Reports*, **41**, 15 pp.
- Bortone, S.A., Kimmel, J.J. & Bundrick, C.M., 1989. A comparison of three methods for visually assessing reef fish communities: time and area compensated. *Northeast Gulf Science*, **10**, 85–96.



- Bussotti, S., Denitto, F., Guidetti, P. & Belmonte, G., 2002. Fish assemblages in shallow marine caves of the Salento Peninsula (Southern Apulia, SE Italy). *P.S. Z.N.I.: Marine Ecology*, **23**, Supplement, 11–20.
- Castellarin, C., Visintin, G. & Odorico, R., 2001. L'ittiofauna della riserva naturale marina di Miramare (Golfo di Trieste, Alto Adriatico). *Annales, Series Historia Naturalis*, **11**, 207–216.
- Clarke, K.R. & Warwick, R.M., 1994. *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth: Natural Environment Research Council, UK.
- Côté, I.M., Mosquera, I. & Reynolds, J.D., 2001. Effects of marine reserves characteristics on the protection of fish populations: a meta-analysis. *Journal of Fish Biology*, **59**, 178–189.
- D'Anna, G., Lipari, R., Badalamenti, F. & Cuttitta, A., 1999. Questions arising from the use of visual census in natural and artificial habitats. In *Fish visual census in marine protected areas* (ed. M. Vacchi et al.). *Il Naturalista Siciliano*, **23**, Supplement, 187–204.
- Dayton, P.K., Sala, E., Tegner, M.J. & Thrush, S., 2000. Marine reserves: parks, baselines, and fishery enhancement. *Bulletin of Marine Science*, **66**, 617–634.
- De Girolamo, M., Scaggiante, M. & Rasotto, M.B., 1999. Social organization and sexual patterns in the Mediterranean parrotfish *Sparisoma cretense* (Teleostei: Scaridae). *Marine Biology*, **135**, 353–360.
- De Girolamo, M., Stefanni, S., Mazzoldi, C. & Odorico, R., 1998. Effetti della proibizione della pesca sul popolamento ittico del Parco marino di Miramare (TS): analisi preliminare. *Bollettino del Museo Civico di Storia Naturale di Venezia*, **49**, Supplement, 311–315.
- Dulvy, N.K., Polunin, N.C.V., Mill, A.C. & Graham, N.A.J., 2004. Size structural change in lightly exploited coral reef fish communities: evidence for weak indirect effects. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 466–475.
- Fischer, W., Bauchot, M.L. & Schneider, M., 1987. *Fiches FAO d'identification des espèces pour les besoins de la pêche. Méditerranée et Mer Noire. Zone 37. II Vertébrés*. Rome: FAO.
- Francour, P., 1994. Pluriannual analysis of the reserve effect on ichthyofauna in the Scandola natural reserve (Corsica, Northwestern Mediterranean). *Oceanologica Acta*, **17**, 309–317.
- García-Charton, J.A., Pérez-Ruzafa, A., Sánchez-Jerez, P., Bayle-Sempere, J.T., Reñones, O. & Moreno, D., 2004. Multi-scale spatial heterogeneity, habitat structure, and the effect of marine reserves on Western Mediterranean rocky reef fish assemblages. *Marine Biology*, **144**, 161–182.
- García-Rubies, A. & Zabala, M., 1990. Effects of total fishing prohibition on the rocky fish assemblages of Medes island marine reserve (NW Mediterranean). *Scientia Marina*, **54**, 317–328.
- Glasby, T.M., 1997. Analysing data from post-impact studies using asymmetrical analyses of variance: a case study of epibiota on marinas. *Australian Journal of Ecology*, **22**, 448–459.
- Guidetti, P., 2000. Differences among fish assemblages associated with nearshore *Posidonia oceanica* beds, rocky-algal reefs and unvegetated sand habitats in the Adriatic Sea. *Estuarine, Coastal and Shelf Science*, **50**, 515–529.
- Guidetti, P., 2002. Mediterranean MPAs: the importance of experimental design in detecting the effects of protection measures on fish. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **12**, 619–634.
- Guidetti, P., 2004. Fish assemblages associated with coastal defence structures in south-western Italy (Mediterranean Sea). *Journal of the Marine Biological Association of the United Kingdom*, **84**, 669–670.
- Guidetti, P., Bussotti, S. & Boero, F., 2005. Evaluating the effects of protection on fish predators and sea urchins in shallow artificial rocky habitats: a case study in the northern Adriatic Sea. *Marine Environmental Research*, **59**, 333–348.
- Guidetti, P., Terlizzi, A., Fraschetti, S. & Boero, F., 2003. Changes in Mediterranean rocky-reef fish assemblages exposed to sewage pollution. *Marine Ecology Progress Series*, **253**, 269–278.
- Halpern, B.S., 2003. The impact of marine reserves: do reserves work and does reserve size matter? *Ecological Applications*, **13**, 117–137.
- Harmelin, J.G., 1987. Structure et variabilité de l'ichtyofaune d'une zone rocheuse protégée en Méditerranée (Parc national de Port-Cros, France). *P.S. Z.N.I.: Marine Ecology*, **8**, 263–284.
- Harmelin, J.G., Bachet, F. & Garcia, F., 1995. Mediterranean marine reserves: fish indices as tests of protection efficiency. *P.S. Z.N.I.: Marine Ecology*, **16**, 233–250.
- Harmelin-Vivien, M.L. et al., 1985. Evaluation des peuplements et populations de poissons. Méthodes et problèmes. *Revue Ecologie (Terre Vie)*, **40**, 467–539.
- La Mesa, G. & Vacchi, M., 1999. An analysis of the coastal fish assemblage of the Ustica Island Marine Reserve (Mediterranean Sea). *P.S. Z.N.I.: Marine Ecology*, **20**, 147–165.
- Macpherson, E., Gordo, A. & Garcia-Rubies, A., 2002. Biomass size spectra in littoral fishes in protected and unprotected areas in the NW Mediterranean. *Estuarine, Coastal and Shelf Science*, **55**, 777–788.
- Micheli, F., Benedetti-Cecchi, L., Gambaccini, S., Bertocci, I., Borsini, C., Osio, G.C. & Romano, F., in press. Alternate states, marine protected areas, and the structure of Mediterranean rocky-reef assemblages. *Ecological Monographs*.
- Mosquera, I., Côté, I.M., Jennings, S. & Reynolds, J.D., 2000. Conservation benefits of marine reserves for fish populations. *Animal Conservation*, **4**, 321–332.
- National Research Council, 2001. *Marine Protected Areas—tools for sustaining ocean ecosystems*. Washington, DC: National Academy Press.
- Ota, D. & Odorico, R., 1993. Osservazioni preliminari sull'attività dei pesci di scogliera nella Riserva Marina di Miramare. *Biologia Marina*, **1**, Supplement, 215–216.
- Underwood, A.J., 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3–15.
- Underwood, A.J., 1997. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge: Cambridge University Press.
- Vacchi, M., Bussotti, S., Guidetti, P. & La Mesa, G., 1998. Study on the coastal fish assemblage in Ustica Island's Marine Reserve (Southern Tyrrhenian Sea). *Italian Journal of Zoology*, **65**, 281–286.
- Vacchi, M. & Tunesi, L., 1993. Stationary visual census: a technique for the assessment of fish assemblages in Mediterranean protected coastal areas. *Bollettino di Oceanografia Teorica ed Applicata*, **11**, 225–229.
- Verginella, L., Spoto, M., Ciriaco, S. & Ferrero, E., 1999. Ethogram of the reproductive territorial male of the Mediterranean damselfish *Chromis chromis* L. (Pisces: Pomacentridae). *Bollettino della Società Adriatica di Scienze*, **78**, 437–454.
- Vigliola, L. et al., 1998. Spatial and temporal patterns of settlement among sparid fishes of the genus *Diplodus* in the northwestern Mediterranean. *Marine Ecology Progress Series*, **168**, 45–56.
- Willis, T.J., Millar, R.B., Babcock, R.C. & Tolimieri, N., 2003. Burdens of evidence and the benefits of marine reserves: putting Descartes before des horse? *Environmental Conservation*, **30**, 97–103.

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